STUDY OF THE RENAL-KALLIKREIN-KININ SYSTEM IN ACUTE POSTISCHEMIC RENAL FAILURE

by

T. NÁDRA

Eötvös University, Department of Biochemistry Budapest

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Summary

This paper deals with the study of the renal kallikrein and total kininase activities in rats with acute renal failure (ARF). The kidneys were homogenized and the homogenates were fractionated by differential centrifugation. The activity of kallikrein was assayed in the microsomal fractions using TAME (4-tolluenesulfonylarginine methyl ester) substrate. Total kininase activity was determined in the homogenate and isolated brush border fraction using bradykinin substrate in a rat uterus *in vitro* bio-assay system. The renal kallikrein activity was found to decrease in acute experimental postischemic renal failure. Kininase activity was reduced due to the damage of the brush border, thus extrarenal kinins could enter the distal tubules and inhibit the activity of kallikrein by end-product inhibition.

Introduction

The kallikrein-kinin system (KKS) is present in the blood circulation and in various organs. First of all it consists of bioactive peptides and proteases wich generate and inactivate these peptides. The name kallikrein designates a group of proteases which release kinin from kininogen by limited proteolysis. Of the two forms plasme kallikrein is present as inactive precursor whereas galndular kallikreins are usually present as active molecules in salivary glands and kidneys, but in the pancreas and intestines the inactive, prekallikrein form also occurs.

The renal kallikrein is bound to the plasma membrane and 90% of its activity is confined to the cortex. As it was shown by S c i c l e et al. [1] the enzyme enters the nephron at the level of the distal tubules. C a r o n e et al. [2] and S c i c l e et al. [3] have demonstrated that the kallikrein-generated active bradykinin molecules are degraded in the proximal tubule and their newly synthesized forms reappear in the distal tubules. Thus a kinin degrading system protects the kidney against the pharmacological actions of kinins filtered through the glomeruli.

The third components of the KKS are the kininases which inactivate the kinins by proteolysis. Of particular importance is kininase II that is primarily involved in the inactivation of kinins. [4] Results of studies conducted with inhibitors of kininase II appear to suggest that the KKS plays a role in water and electrolyte transport at the level of the distal tubules. [5] The target of its action is either directly the distal Na^+ transport and/or the intrarenal blood flow redistribution.

The acute renal failure (ARF) is currently believed to be a syndrome. It is interesting to note that drinking of physiological saline for a few days makes the animals resistant to the development of either ischemic [6] or nephrotoxic acute renal failure [7]. Since the KKS is involved in the control of salt and water metabolism and because during pancreas necrosis the proteases of the gland are activated therefore the purpose of the present investigations was to study the alterations in the KKS during ARF.

Materials and Methods

Male albino rats with an average body weight of 240 g were used. Under nembutal anaesthesia both kidneys were exposed through a lower midline abdominal incision. The renal arteries in both sides were occulded using metal clips. The wound was transiently closed and the animals were placed on thermostatted cushion for 60 min. Then the bilateral occlusion was released and the wound closed by sutures. Controls were subjected to sham-operation. Twenty four hours later blood urea-N concentrations were determined (REANAL test-kit) and the kindeys were removed and weighed (Table I).

Tissue kallikrein activity was assayed in the microsomal fraction isolated according to the method of Ward et al. [8] using 4-toluenesulfonylarginnin methyl ester (TAME) sunstrate. Since the renal kininase activity resides ib the brush border of the proximal tubules [2], [3], therefore the kininase activity was determined in the brush border fraction isolated as described by W i l f o n g – N e v i l l e [9]. Alkaline phosphatase was as used as marker enzyme. Kininase activity was determined by the method of W a r d [8]. The principle of this method is that following the incubation of bradykinin with aliquots of the brush vorder fraction the remaining substrate is bioassayed in an *in vitro* smooth muscle (uterus) preparation.

The activity of alkaline phosphatase was determined using β-glycerolphosphate substrate [10]. Activities in all instances were expressed for protein determined according to the method of L o w r y et al .[11].

Results and Discussion

Data in Table I illustrate that our method was suitable to induce the development of shock-kidney; the characteristic laboratory finding – augmented serum levels of urea – was observed.

The increase of the relative activity of alkaline phosphatase – as a marker for the microsomal and brush border fractions – compared to that

of the homogenate indicates the purity of the fractions. It appears that the purity of the microsomal and brush border fractions was satisfactory for studies of membrane-bound enzymes (Table II). (The level of significance in conformity with data published [8, 9] was 10%.

Table I

Changes in kidney weight and serum urea nitrogen concentration

Group	Kidney weight (g)	Serum Urea-N (mmol/L)
Control	0.36 ± 0.08	6.63 ± 1.23
Acute Renal Failure	0.58 ± 0.15	55.9 ± 4.76

Data are means ± S. D. of 12 – 12 animals

Table II

Changes in renal alkaline phosphatase activity*

Group	Homogenate	Microsomal fraction	Brush Border	Relative Activity
Control	88.3 ± 19.5	308.8 ± 97.2	–	3.49
Acute Renal Failure	75.8 ± 22.0	431.81 ± 113.6	–	5.69
Control	136.0 ± 12.7	–	557.3 ± 108.0	4.09
Acute Renal Failure	52.4 ± 17.2	–	152.9 ± 34.2	2.91

* Activities are expressed in umol Pi/mgproth/h
Data are means ± S. D. of 12 – 12 animals

Alkaline phosphatase activites of the homogenate, microsomal and brush border fractions were compared between control and experimental animals (Table II) in order to detect the possible effects of ARF at this level, too. No alternations were seen in the activity of the microsomal fraction. The alkaline phosphatase activity in both the crude homogenate and brush border fraction obtained from rats with ARF was lower than control. This difference is attributed to the effect of the ARF. No differences were noted in the kallikrein activity of the homogenates prepared from control or experimental rats (Table III). The relative specific activity of control microsomal fraction was 6.58, whereas that of the postischemic microsomal fraction was 3.25. This may suggest a pathological intrarenal distribution of kallikrein thus the possibility of autodigestion should also be borne in mind. The ARF resulted in a reduction of kallikerein activity without affecting the structure of the membran to which the enzyme is bound.

Table III

Changes in renal kallikrein activity*

Group	Homogenate	Microsomal fraction	Relative activity
Control	0.85 ± 0.24	5.06 ± 1.12	6.58
Acute Renal Failure	0.85 ± 0.24	2.90 ± 0.89	3.25

* Activities are expressed in umol TAME/mgprot/h
Data are means ± S. D. of 12 – 12 animals

Table IV

Changes in renal kininase activity*

Group	Homogenate	Brush border	Relative activity
Control	2.70 ± 0.98	28.76 ± 4.31	10.65
Acute Renal Failure	3.79 ± 1.13	7.78 ± 1.55	2.05

* Activities are expressed in ug bradykinin/mgprot/h
Data are means ± S. D. of 12 – 12 animals

The kininase activity of the brush border fraction isolated from rats with ARF was substantially lower than is control (Table IV.) This finding and the similar but less apparent diminution of alkaline phosphatase activity appear to suggest that the brush border membrane was damage d in ARF. We should, however, note that only total kininase activity was determined in the kidneys since the specific substrate (hippuryl-glycine) of the kininase II was not available. Thus we are not able to tell the contribution of kininase I and kininase II to total kininase activity. However, based on the source of the preparation we presume that the activity measured was kininase II and possible activity of kininase I from extrarenal sources was negligible.

In conclusion we suggest that the activity of kininase II in the brush border of the proximal tubules was diminished in ARF depending on the extent of the membrane damage. Thus the kinins of extrarenal origin can "readily" enter the distal tubules where they elicit dramatic changes in electrolyte and water metabolism leading to natriuresis, hyperkalemia, etc. The kinins from extrarenal sources might inactivate the plasma membrane-bound kallikrein by end-product inhibition at the level of the distal tubules. However, detailed enzyme kinetic studies are required to prove this hypothesis.

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